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Short Communication

In vitro skin whitening and lightening properties of Sri Lankan low grown orthodox Orange Pekoe grade black tea

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ABSTRACT

Presently, there is a demand for development of cheap, safe and effective skin whitening and lightening herbal cosmeceutical formulations. In this connection, this study, was performed to assess the skin whitening and lightening properties of Sri Lankan low grown orthodox Orange Pekoe (O.P) grade of black tea (made from buds and upper most tender leaves of *Camellia sinensis* L. plant), by determining its inhibiting potential of the activity of mushroom tyrosinase enzyme *in vitro*. The concentrations tested were 25, 50, 100, 200 and 400 µg/ml and reference drug used was quercetin. The results showed that Sri Lankan O.P. grade black tea has mild but dose-dependent antityrosinase activity *in vitro*. This is a new finding for any grade of Sri Lankan black tea. It is concluded that Sri Lankan O.P. grade black tea has the potential to be developed as a cheap, safe and effective multipurpose (antioxidant, sun screening or antiaging) skin whitening and lightening herbal cosmeceutical (considering its other bioactivities reported).

Key words: Black tea, Orange Pekoe, skin whitening, skin lightening cosmeceuticals, *Camellia sinensis*

INTRODUCTION

Women's desire for white skin is universal although there are cultival variations [1]. In Asian countries, there is a long history of using skin whitening and lightening cosmeceutical formulations [1]. These are still popular not only in Asian countries, but in other non-white cultures as well [1]. The global market for skin lightners is projected to reach US $ 19.8 billion by 2018, based on sales growth in Asia, Africa and Middle East alone [2]. Although, there are several skin whitening and lightening tropical skin formulations available in the market today, [2,3] their skin application often induce several unpleasant side effects depending on the product: overpigmentation; thinning of skin, so called "black panda effect" ; becoming more sensitive to UVA and UVB rays of the sun; bleeching of skin; allergies; contact dermatitis ;conjunctivitis; skin irritation; permanent depigmentation giving the appearance of a person with vitiligo; and increasing the risk of skin cancer [2,3]. Further, most lack the claimed potencies, their long term effects are unknown and also the ones manufactured in Europe are expensive [3]. Hence, there is an imperative need for the development of novel effective, cheap and safe cosmaceuticals as skin whiteners and lightners, preferably from herbal sources: the demand for herbal cosmeceutical medications are rising exponentially [3]. In this regard, this study was launched to assess the skin whitening and lightening potential of Sri Lankan low grown orthodox Orange Pekoe (O.P) grade black tea by determining its inhibitory potential of tyrosinase enzyme, *in vitro*. In Sri Lanka, as a home remedy, black tea brews are applied on to hyperpigmented areas in the infra orbital regions of the face.

MATERIAL AND METHODS

Sources of tea: Top most immature leaves and buds of *Camellia sinensis* L. plucked from the plantation of St. Jochims tea estate of the Tea Research Institute, Hedallana, Ratnapura, Sri Lanka (29 m above mean sea level: low grown) during November-December 2011 were used to process the O.P. grade black tea by orthodox-rotovane technique at the estate factory. The sieve analysis of the sample has shown that 83.5 % of tea

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particles were true sized (1400 – 2000 µm) and typical for the grade [4]. Further organoleptic profile analysis made by the professional and experienced tea tasters at the Tea testing unit of Sri Lankan Tea Board has confirmed that the sample used can be accepted as well made high quality low grown O.P. grade Sri Lankan black tea [4]. Tea samples were packed in triple laminated aluminium foil bags (1 kg each) and stored at -20^0 C until use.

Preparation of black tea brew (BTB): BTB was made according to the ISO standards (ISO 3103): by adding 2 g of O.P. grade black tea to 100 ml of boiling water and brewed for 5 min [5]. This contained 36.1 % (w/v) tea solids in water. BTB was freeze dried and different concentrations (25, 50, 100, 200 and 600 µg/ml) were made by dissolving appropriate weights of the freeze dried tea solids in isotonic saline (0.9 % NaCl, w/v).

Evaluation of anti-tyrosinase activity of black tea brew: Tyrosinase enzyme inhibitory activity of O.P grade black tea (n = 4) extract was carried out according to the method describe by Masuda, *et al* 2005 [6] with some modifications using L-DOPA as the substrate. A reaction volume of 200 µl containing 40 µl of mushroom tyrosinase enzyme (0.25 mg/ml) and different concentrations of black tea extract (assay concentrations 400, 200, 100, 50, 25 µg/ml) were incubated with 0.1 M phosphate buffer pH 6.8, at 37^0 C for 10 minutes. Reaction was started by adding 40 µl of 2.55 mM L-DOPA and absorbance was measured at 475 nm after 10 minutes incubation at 37^0 C. Each sample was accompanied by a blank that had all the components except L-DOPA. Quercetin was used as the standard (n = 4) and its antityrosinase activity was determined using four concentrations (3.125, 6.25, 12.5, 25 µg/ml). Results were compared with the control. The percentage tyrosinase inhibition was calculated as follows:
$(A_{control} - A_{sample}) / A_{control} \times 100$

Statistical analysis: Data is represented as Mean ± Standard error of mean (SEM). IC_{50} values were computed and regression analysis was made using Excel software. Significance was set at $P < 0.05$.

RESULTS

The results obtained are summarized in Tables 1 and 2. As shown, at the concentration range tested, O.P. grade black tea impaired the tyrosinase activity mildly (ranging from 8.71 to 40.68 %). This anti tyrosinase activity was dose- dependent ($r^2 = 0.96$, $P < 0.05$). The mean fifty percent inhibiting concentration, IC_{50} value was 496.15 ± 10.42. Unfortunately, the concentration of tea could not be increased further due to interferences in colour. In contrast, tyrosine inhibitory activity of the reference drug, quercetin, ranged from 8.35 to 83.80 % with a IC_{50} value of 29.38 ± 0.49.

DISCUSSION

This study examined the *in vitro* skin whitening and lightening properties, in terms of tyrosinase inhibition activity of Sri Lankan orthodox low grown O.P. grade black tea. Tyrosinase is the key enzyme involved in the biosynthesis (melanogenesis) of melanin, the colouring pigment of the mammalian skin, including humans [7]. The tea sample used here, unlike in most studies, on the bio activities of black tea, was garden fresh, unblend and typical to the grade and agroclimatic elevation (in terms of sieve analysis and organoleptic properties [4]). Also, the brew was made according to the ISO specification [5], before freeze drying. The *in vitro* assay used to determine anti-tyrosinase activity is simple, well established, validated and generate quick results which are also reproducible [6]. The enzyme used was mushroom tyrosinase, which is highly homologous with the mammalian tyrosinase [7]. However, it should be noted that the mushroom tyrosinase is a cytosol enzyme while human tyrosinase is a membrane bound enzyme located in the melanosomes, specialized organelles, found in melanocytes [7, 8]. In addition, mushroom tyrosinase is a tetramer, in contrast, to the monomer type of the human enzyme [7]. Interestingly, todate, no tyrosinase inhibitory studies have been conducted with human tyrosinase due to lack of purified enzyme [7]. However, in spite of these facts, some have extrapolated their data with mushroom tyrosinase in to human situation [7].

The results demonstrate that Sri Lankan low grown orthodox O.P. grade black tea can mildly inhibit tyrosinase activity has (anti-tyrosinase activity) *in vitro*. Unfortunately, it was not possible to increase the concentration of black tea further (beyond 400 µg/ml) due to colour interferences. However, this sort of mild inhibition of tyrosinase is desirable in the context of developing a skin whitening agent since its strong inhibition can lead to vitiligo like condition. This is a novel finding showing the skin whitening and lightening potential of Sri Lankan O.P. grade black tea. Up to date, only two studies have attempted to evaluate the whitening and lightening potentials of tea: one study with black tea using brown guinea pigs [9] and the other, with both guinea pigs and *in vitro* in B 16 mouse melanoma cells using oolong tea [10].

The anti-tyrosinase activity of O.P. grade tea was dose-related. This experimental observation indicates a genuine and a specific effect mediated

via suppression in tyrosinase activity: rather than through a non specific enzyme inactivation due to denaturing of the enzyme, from acidity or alkalinity of the reactive medium, since a powerful buffering system was used. We have previously shown that Sri Lankan O.P. grade black tea contained a variety of flavonoids such as epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin, gallocatechin or catechin and large polymeric polyphenols like theaflavins and thearubigins [11]. Others have shown, the presence of flavonols such as quercetin, myricetin and kaemplerol in Sri Lankan black tea [12]. It is well recognized that polyphenols, flavonoids, flavonols are tyrosinase inhibitors [7]. Usually, these phytoconstituents act as competitive substrate inhibitors of tyrosinase [7]. Thus, the tyrosinase inhibitory activity observed in this study may be attributed to these phytoconstituents of black tea. However, it is worth mentioning that most of the flavonol tyrosinase inhibitors are weak compared to the inhibitory strength of kojic acid [7] which is used in skin whitening formulations [2,3].

Currently, the emerging trend is to incorporate antioxidants and sun screening ingredients in to skin whitening and lightening formulations [2,3], as they boost up skin care value. We have previously shown that Sri Lankan black teas including O.P. grade tea have sun protection factor values higher than fifteen [13]. Dermatologists strongly recommend to apply sun screens having sun protection factor values of fifteen or more to avoid deleterious of effects of suns UVA and UVB rays [13, 14]. Obviously, this sun protection effect of Sri Lankan O.P. grade black tea would enhance its value as a skin whitening agent: the main physiological stimulus for human melanogenesis is UV radiation [7]. Sri Lankan O.P. grade black tea has strong antioxidant activity [15] as other black teas [12]. Infact, tea is the most potent herbal antioxidant [12]. Free radicals are linked with radiation damage of the skin [16] and skin aging [16]. Thus, the antioxidant activity of O.P. grade black tea further strengthens its potential as a tropical skin formulation. Presence of mild anti-elastase activity in O.P. grade tea [17] is yet another positive factor for developing it in to a skin whitening cosmeceutical. What is more, Sri Lankan O.P. grade tea has shown to possess antiglycation and AGEs cross-link breaking activities [18]. Since, advanced glycocelated end products (AGEs) are linked with premature aging of skin [19] the presence of these two activities would remarkably potentiate its use as a skin whitener.Tea is the most consumed beverage besides water [12]. Thus, possibility exists that regular drinking of black tea could act as a supplementary beverage to whiten human skin. However, further animal experiments and clinical trials are needed before firm recommendations are made, although tea is a well known safe beverage [11]. It is hoped that the findings of this study may prompt cosmeceutical manufacturers to add Sri Lankan black tea to their skin care products.

Another aspect of this study is that, it shows the potential of black tea as a food anti-browning naturceutical: browning reaction in damaged fruits during post-harvest handling and processing primarily mediated via tyrosinase [7]. Further in depth studies are, however, warrented in this aspect.

CONCLUSION

This study shows, for the first time, that Sri Lankan low grown orthodox O.P. grade black tea has mild tyrosine inhibitory activity *in vitro* when tested using mushroom tyrosinase enzyme. This property taken together with its antioxidant, sun screening, anti elastase, anti glycation and AGEs cross-bridge braking activities suggests its promise as a skin whitening and lightening cosmeceutical and a systemic supplementary beverage to promote skin health.

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Table 1: Anti-tyrosinase activity of Sri Lankan Orange Pekoe grade black tea

Concentration (µg/ml)	% Inhibition
400	40.68 ± 0.59
200	28.52 ± 0.70
100	19.32 ± 0.64
50	14.16 ± 1.26
25	8.71 ± 0.16

(Data presented as Mean ± SEM, n = 4)

Table 2: Anti-tyrosinase activity of quercetin

Concentration (µg/ml)	% Inhibition
50	83.80 ± 0.89
25	44.67 ± 1.24
12.5	19.42 ± 0.23
6.25	11.09 ± 0.57
3.125	8.35 ± 0.73

(Data presented as Mean ± SEM, n = 4)

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